SPECIFIC INTERACTION OF COBALTIC COMPLEXES WITH MYOSIN

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1. Introduction

The protein myosin is rather unique as its molecules consist of both globular and rodlike parts. The former contains two 'heads' (HMM S-1) each of which can bind actin and split ATP – apparently at the same rate [1–2]. Preparations of isolated heads have recently been found to be capable of generating a mechanochemical force by interacting with actin filaments in the presence of ATP. It was therefore concluded that neither the aggregation of myosin molecules into filaments (via the water-insoluble rods of light meromyosin, (LMM)) nor the concerted action of two bound-together heads is indispensable for mechanochemical energy transduction [3–6]. These findings do not, however, solve the problem 'why two heads' or exclude the possibility that the two heads differ from each other in some respects and/or that cooperative effects between the two heads play a role in motile systems under physiological conditions.

It has recently been demonstrated [7,8] that a myosin–ATP–Mg^{2+} complex is stabilized in an activated conformation before the products of ATP hydrolysis, ADP and inorganic phosphate, are released. The process of contraction which involves the actin filaments is likely to take place during this activation step. It makes sense to believe that the rodlike parts of the myosin molecule i.e., HMM subfragment-2 and LMM are informed of the state of the catalytic site and, vice versa, that the 'contractile complex' is affected by the state of the rodlike parts. The latter could differ according to whether LMM is present or absent (as in HMM or HMM S-1) and/or according to the level of aggregation of myosin.

An indication for a possible effect of LMM on the catalytic activity of myosin was recently found. We have shown [9] that affinity labeling of one of myosin's active sites with Co^{3+}-phenanthroline-ATP, [Co-(phen)-ATP] while abolishing the ATPase activity of that site, enhances remarkably the activity of the second site; moreover, the rate of labeling of the latter is much smaller than that of the first. These effects were, however, absent upon labeling of heavy meromyosin (HMM). Since the labeling was performed at a low ionic strength, it was interesting to repeat these experiments under conditions at which myosin does not aggregate into filaments (i.e. at a high ionic strength). The idea was to find out whether the difference in behavior between myosin and HMM originates from the very existence of LMM in the former or is associated with aggregation. In addition, we tested the ability of this cobaltic complex to label HMM S-1. In our previous study we have also found that several other cobaltic complexes could inhibit myosin ATPase. Since cobaltihexammine very often acts as a Ca^{2+} analog ([10]; also Danchin, unpublished data) we have investigated the influence of this complex on the Ca^{2+}- and the EDTA-ATPase activities of myosin.

2. Materials and methods

2.1. *Cobaltic complexes*

Co-(phen)-ATP was prepared as in ref. [9] and further purified by ethanol precipitation (0.8–1.0 v/v) at −20°C. This complex most probably contains also the ion O_2^- (its structure will be presented elsewhere). Cobaltihexammine was prepared according to Bjerrum [10].

The affinity labeling of myosin and of HMM S-1 was carried out as in [9] in media containing various KCl concentrations.

2.2. *Myosin and HMM S-1*

Myosin was obtained [11] from the white back muscles of New Zealand white rabbits and stored at −20°C in 50% glycerol. HMM S-1 was prepared from myosin [12] and further purified by ATP affinity chromatography columns [2,13]. ATPase activities were determined as described previously [9]. Inhibition by cobaltihexammine was followed by adding various concentrations of the complex to the ATPase assay solutions which contained 10 μM to 5 mM ATP.

3. Results

3.1. *Affinity labeling with Co-(phen)-ATP*

Myosin was labeled with 0.96 mM Co-(phen)-ATP in the presence of 0.6 M KCl. For comparison, experiments were also repeated at a low ionic

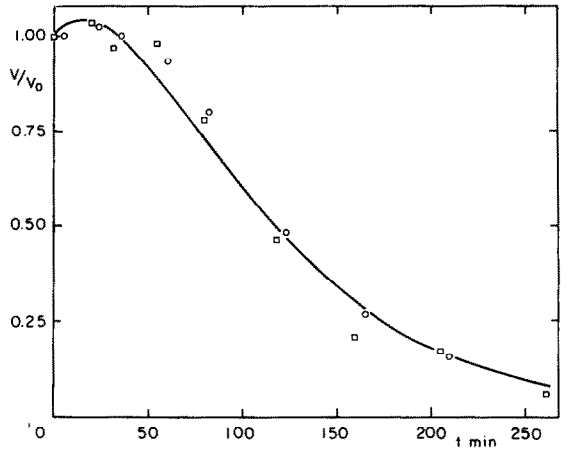


Fig. 1. Labeling of dispersed myosin. Myosin is incubated at 0°C in a medium containing 5 mM potassium phosphate pH 7.0, KCl 0.60 M and 0.96 mM of Co-(phen)-ATP. Aliquots are assayed at different times for Ca-ATPase (circles) and EDTA-ATPase (squares). The solid line is a *theoretical* curve representing the equation [9]:

$$V/V_0 = \frac{((s\text{-}1)c_0k_1 + \lambda_2)\, e^{-\lambda_1 t} - ((s\text{-}1)c_0k_1)\, e^{-\lambda_1 t}}{\lambda_2 - \lambda_1}$$

$$\text{with } \lambda_1 = \frac{c_0k_1 - \sqrt{\Delta}}{2};\ \lambda_2 = \frac{c_0k_1 + \sqrt{\Delta}}{2}$$

$$\sqrt{\Delta} = c_0k_1\sqrt{1 - 4\frac{k_2}{k_1}}$$

$s = 1.18$

$k_1 = 0.594\ M^{-1}\ sec^{-1}$; $k_2 = 0.148\ M^{-1}\ sec^{-1}$

Table 1
Kinetic parameters of the affinity labeling by Co(phen)-ATP of myosin, HMM and HMM S-1

		Myosin			HMM[a]	HMM S-1
KCl	(M)	0.60	0.15	0.12[a]	0.12	0.15
s		1.18	1.40	1.75	1.0	1.0
$k_1/2$	(M^{-1} sec^{-1})	0.297	0.305	0.311	k = 0.045 (M^{-1} sec^{-1})	k = 0.084 (M^{-1} sec^{-1})
k_2	(M^{-1} sec^{-1})	0.148	0.107	0.119		

$M + C \overset{k_1}{\rightarrow} MC$; $MC + C \overset{k_2}{\rightarrow} MC_2$, where M denotes my osin or myosin subfragment and C is Co(phen)-ATP.

[a] From ref. [9].

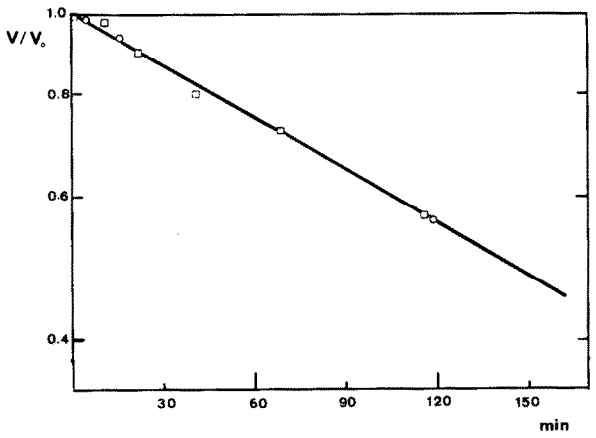
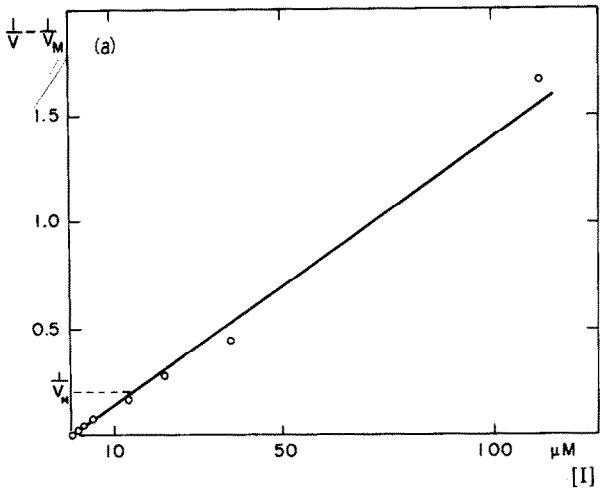


Fig. 2. Labeling of heavy meromyosin S-1. Conditions as in fig. 1, except that the KCl concentration was 0.15 M.



strength (0.15 M KCl). Fig. 1 shows the ATPase activity as a function of the incubation time at high ionic strength. Both Ca^{2+}- and EDTA-activated ATPases increase slightly at first and then decrease down to zero. The initial enhancement was very small in comparison with that observed at low ionic strengths (see fig. 2 in ref. [9]). The results of the kinetic analysis [9] are summarized in table 1. The most prominent effect of the high salt concentration is the decrease in the value of *s*, defined as the enhancement in the activity of a myosin molecule labeled at one active site. In accordance with our previous observation, inhibition could be abolished by the addition of thiol reagents such as dithiothreitol.

HMM S-1 could also be labeled with Co-(phen)-ATP (fig.2). Inactivation followed simple first-order kinetics with respect to the active sites concentration.

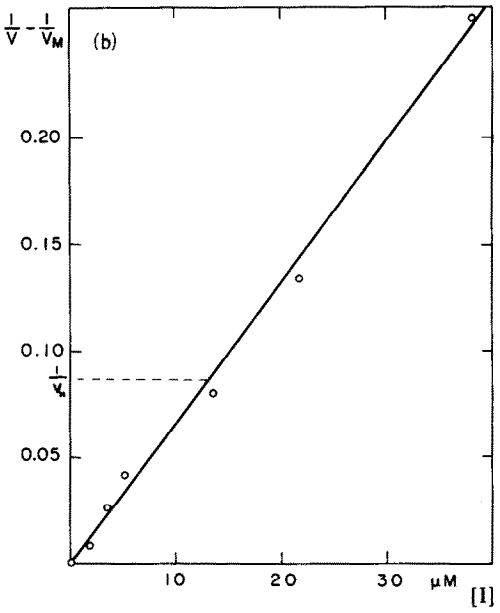


Fig. 3. Inhibition of cobaltihexammine. (a) Inhibition of Ca^{2+}-ATPase activity. Each point represents the intercept with the abcissa of the straight line obtained on plotting V versus 1/[ATP] at different concentration of the inhibitor (where V is the rate in μmoles/min/mg myosin). The assay medium contained about 0.15 mg of myosin, 4 mM $CaCl_2$, 50 mM KCl. Rates were determined in a pH-stat with 0.01 N NaOH as a titrant. B. Inhibition of EDTA-ATPase activity. Same conditions as in fig. 3A, except that the assay medium contained 1 mM EDTA (instead of 4 mM $CaCl_2$) and 500 mM KCl.

3.2. *Inhibition by cobaltihexammine*

When this complex was added to a myosin ATPase assay medium, a strong inhibition was observed. The latter was not affected by EDTA: fig. 3 shows that the value of the inhibition constant K_i was essentially the same for both assay media and that inhibition was non-competitive (K_i = 13-15 μM).

4. Discussion

The kinetic data presented in the previous paper suggested that a half-labeled myosin molecule could exhibit a higher ATPase activity than an unreacted one, even though the activity was completely abolished in the double-labeled myosin. Both the ATPase activity of an unreacted site of myosin and its readiness to bind the Co-(phen)-ATP complex were found to be affected by the labeling of its twin subfragment.

The present work demonstrated that the inactivation pattern is strongly influenced by the ionic strength at which labeling is performed: at high ionic strength, when practically all of the myosin exists in a dispersed state, the site–site interaction is markedly diminished as expressed by the fall in the value of s from an average of 1.75 (for the Ca^{2+}- and the EDTA-activated ATPase activity) to a value of 1.18 (see table 1).

In view of the fact that in living striated muscles a large part of the myosin is present in the form of filaments [3], our observation that site–site interaction in Co-(phen)-ATP half-labeled myosin is much more pronounced in aggregated than in dispersed myosin may suggest another role to filament formation by myosin: to enable some kind of 'information transfer' from one head to its twin via the tails.

Labeling by Co-(phen)-ATP was shown to occur in HMM S-1 as well as in HMM. However, in both these cases there was no enhancement preceding the inactivation. In the case of HMM S-1, which contains only one active site, this behavior is not unexpected. However a similar behavior in the case of HMM, which consists of two myosin heads, seems to indicate that site–site interaction is lost together with LMM.

With regard to the effect of cobaltihexammine, the non-competitive inhibition seems to indicate that the reaction occurs outside the active site. The complex could thus act at the Ca^{2+}-binding sites of myosin which contain the DTNB light chains [14–16]. As shown previously [16], the destruction of this metal ion site by removal of the DTNB light chains can affect the EDTA- and the actin-activated ATPase activity, as well as the regulation by Ca^{2+} of troponin–tropomyosin-containing actomyosin ATPase activity. It should therefore not be surprising that the Ca^{2+}-analog cobaltihexammine, by reacting at the metal ion site, might inhibit the ATPase activity of myosin.

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